Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 7834–7838, August 1995
Cell Biology

Multiple Bcl-2 family members demonstrate selective dimerizations with Bax

THOMAS W. SEDLAK*†, ZOLTÁN N. OLTVAI*†, ELIZABETH YANG*, KUN WANG*, LAWRENCE H. BOISE‡, CRAIG B. THOMPSON‡, AND STANLEY J. KORSMEYER*

*Division of Molecular Oncology, Department of Medicine and Pathology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110; and ‡Howard Hughes Medical, Institute and Department of Medicine, Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

Communicated by Emil R. Unanue, Washington University School of Medicine, St. Louis, MO, May 5, 1995

ABSTRACT **A family of Bcl-2-related proteins regulates cell death and shares highly conserved BH1 and BH2 domains. BH1 and BH2 domains of Bcl-2 were required for it to heterodimerize with Bax and to repress apoptosis. A yeast two-hybrid assay accurately reproduced this interaction and defined a selectivity and hierarchy of further dimerizations. Bax also heterodimerizes with Bcl-x_L, Mcl-1, and A1. A Gly-159 → Ala substitution in BH1 of Bcl-x_L disrupted its heterodimerization with Bax and abrogated its inhibition of apoptosis in mammalian cells. This suggests that the susceptibility to apoptosis is determined by multiple competing dimerizations in which Bax may be a common partner.**

Bcl-2 is present at the t(14;18) chromosomal breakpoint of follicular B-cell lymphoma (1–3). Bcl-2 proved oncogenic because it extended cell survival by inhibiting apoptosis (4–6). An expanding family of Bcl-2-related molecules is most highly conserved in two regions, the Bcl-2 homology 1 and 2 (BH1 and BH2) domains. This includes Bax, a homolog that dimerizes with Bcl-2 and promotes apoptosis (7). Mutagenesis of Bcl-2 indicated that intact BH1 and BH2 domains were required for heterodimerization with Bax. Bcl-2 mutants that failed to heterodimerize with Bax could no longer repress cell death (8). The ratio of Bcl-2 to Bax dictates a cell's susceptibility to an apoptotic stimulus (7). In another system, the ratio of Bcl-2 to Bax appeared to decrease following a death stimulus (9, 10). Thus, homology within BH1 and BH2 principally defines membership in the family and these domains have been shown to regulate heterodimerization. The conservation of BH1 and BH2 suggests that other family members may also regulate cell death through competing dimerizations. However, important questions exist as to whether there is any selectivity of interactions among family members or variation in the strength of dimerizations. To assess this, we utilized a yeast two-hybrid assay and pursued the biologic relevance of a predicted interaction in mammalian cells.

MATERIALS AND METHODS

Yeast Two-Hybrid Analysis. Plasmids were transformed into yeast strain PCY2 (*MATα Δgal4 Δgal180 URA3::GAL1-lacZ lys2-801^{amber} his3-Δ200 trp1-Δ63 leu2 ade2-101^{ochre}*), which harbors the GAL4 upstream activating sequence (UAS_g) with a *lacZ* reporter gene. GAL4 DB fusion proteins bind the UAS_g and *lacZ* transcription is activated from the GAL1 minimal promoter if GAL4 AD and GAL4 DB fusion proteins interact (11). The C-terminal signal-anchor sequences of Bcl-2 family members (12) were removed and cloned into pAS1-CYH2 for GAL4 DB and pACTII for GAL4 AD (13).

Yeast were transformed by a lithium acetate method and grown on appropriate selective medium. After 4–6 days, yeast were transferred to nitrocellulose filters and incubated for 1 min in liquid N_2. Dry filters were incubated overnight with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) substrate solution (60 mM Na_2HPO_4/40 mM NaH_2PO_4/10 mM KCl/1 mM $MgSO_4$/50 mM 2-mercaptoethanol/1 mg of X-Gal per ml, pH 7).

Quantitative *o*-Nitrophenyl β-Galactoside (ONPG) Assay. Briefly, 0.1 ml of yeast transformant extracts was added to 0.9 ml of Z buffer (60 mM Na_2HPO_4/40 mM NaH_2PO_4/10 mM KCl/1 mM $MgSO_4$/38.3 mM 2-mercaptoethanol, pH 7) and warmed to 28°C. Reactions were initiated upon addition of 0.2 ml of ONPG substrate (4 mg/ml) in Z buffer and terminated with 0.5 ml of 1 M Na_2CO_3. β-Galactosidase activity (nmol/min per mg of protein) was calculated using the formula $(1.7 \times OD_{420})/(0.0045 \times \text{volume} \times [\text{protein}] \times \text{time})$ with volume of extract added in ml, [protein] in the extract in mg/ml, and time of reaction in min (14). Reactions were terminated within the linear range of the assay ($OD_{420} < 1.0$). For the purpose of comparison, the β-galactosidase activity of GAL4 DB constructs with GAL4 AD vector alone was normalized to 1.0. Data are averages of two dilutions of extracts from three independent experiments.

Bcl-x_L Mutant and Stable Transfected Clones. The single amino acid, WAR substitution, mI-3, was placed in Bcl-x_L by PCR-mediated site-directed mutagenesis. An *Eco*RI fragment containing the full-length human Bcl-x_L cDNA (15) was used as a template in two separate PCRs incorporating a unique *Hpa* I site. The two PCR inserts were cloned into a Bluescript vector and then melded utilizing the unique *Hpa* I site in each insert. Both constructs were sequenced to ensure that only specific positions were modified. The mutated and wild-type Bcl-x_L molecules were cloned into the pSFFV-Neo vector (16) and transfected as described (7). Neomycin-resistant clones were picked at random and examined for expression of human Bcl-x_L by Western immunostaining.

Anti-Bax Monoclonal Antibody (mAb). A mAb was produced in Armenian hamsters using murine BaxΔC19 protein as an immunogen (17). Bax was expressed in *Escherichia coli* strain XL1-Blue (Stratagene) using expression vector plasmid pGex-KG (18). The glutathione *S*-transferase (GST)-Bax fusion protein was captured on GST-agarose beads and cleaved by thrombin, and released Bax protein was concentrated prior to injection.

Immunoprecipitation. Immunoprecipitations were as described (7). Briefly, equal amounts of cells per sample ($5–10 \times 10^6$ cells) were labeled overnight with 100 μCi of [^{35}S]methionine/[^{35}S]cysteine (Tran^{35}S-label; ICN; 1 Ci = 37 GBq). Cell lysates were generated with 0.25% Nonidet P-40 buffer or in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: mAb, monoclonal antibody; Ab, antibody; ONPG, *o*-nitrophenyl β-galactoside; wt, wild-type; HA, hemagglutinin; IL-3, interleukin 3.

†T.W.S. and Z.N.O. contributed equally to this report.

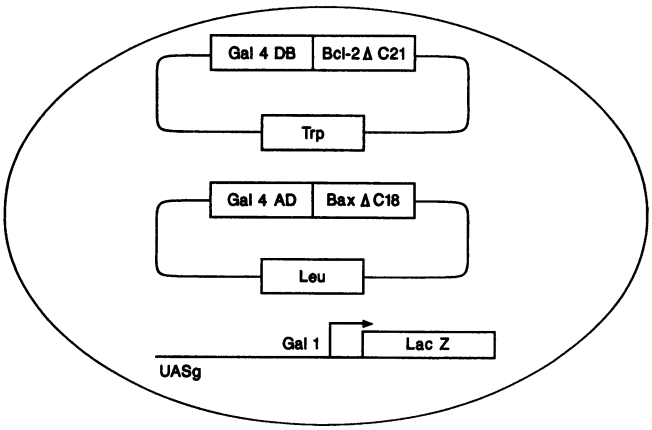

FIG. 1. Yeast two-hybrid schematic. Bcl-2 gene family members were cloned in-frame into a GAL4 DNA binding domain vector (GAL4 DB) and a GAL4 activation domain vector (GAL4 AD).

indicated lanes with RIPA buffer (19), which disrupts Bcl-2/Bax and Bcl-x_L/Bax interactions. Immunoprecipitations were conducted using the indicated Abs followed by protein-A Sepharose bead capture. Immunoprecipitates were electrophoresed through 12.5% SDS/polyacrylamide gels and processed for fluorography.

Western Blot Analysis. Staining procedures were performed in phosphate-buffered saline with 0.05% Tween 20 (PBS/Tween) at room temperature. Filters were incubated with the rabbit anti-Bcl-x polyclonal Ab (1:500), followed by biotinylated goat anti-rabbit Ab (1:330), both for 2 hr. Immunoblots were allowed to react with horseradish peroxidase (HRP)-streptavidin (1:1000) (Zymed) for 1 hr and developed with diazobenzidine (Bio-Rad) enhanced with nickel chloride (0.03%). For Bax immunostaining, filters were incubated with the 4D2 mAb (1:250), followed by biotinylated goat anti-hamster antibody (1:2000), both for 2 hr. Immunoblots were allowed to react with HRP-streptavidin (1:20,000) (Zymed) for 1 hr and developed with enhanced chemiluminescence (Amersham).

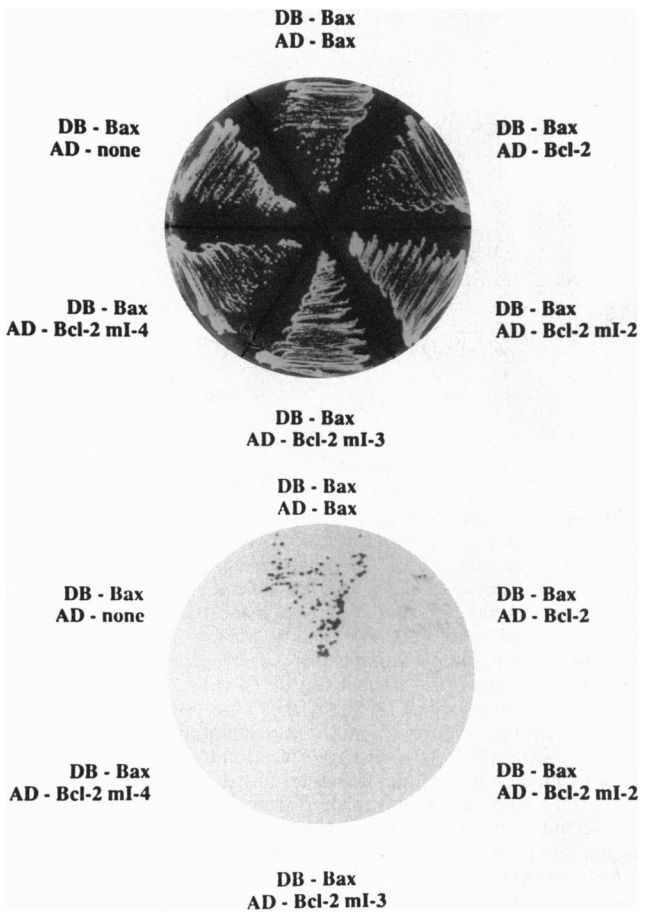

FIG. 2. The yeast two-hybrid system correctly identifies Bcl-2/Bax interactions observed in mammalian cells. Interaction of Bax with Bcl-2 but not the BH1 domain mutants of Bcl-2 mI-2, mI-3, or mI-4.

RESULTS

To explore whether a selectivity and rank order of dimerizations exist among Bcl-2 family members, we established a yeast two-hybrid assay (11). Signal-anchor sequences (12) were removed from Bcl-2 and Bax to ensure their translocation to the nucleus as fusion proteins with the GAL4 DNA binding domain (DB) or the GAL4 activation domain (AD) (Fig. 1). Bax heterodimerized with Bcl-2 to activate transcription of *lacZ* (Fig. 2). Moreover, a Bax/Bax interaction was even stronger, reflecting the relative strengths noted in mammalian cells (7). To further validate this assay, the WGR (amino acids, 144–146) within BH1 of Bcl-2 was altered to AAA (mI-2), WAR (mI-3), or WER (mI-4). These Bcl-2 mutants failed to heterodimerize with Bax in the yeast two-hybrid system, correctly reflecting the disrupted Bcl-2/Bax interactions seen in mammalian cells (Fig. 2) (8). However, as has been noted in mammalian cells, they retain the capacity to interact with wild-type (wt) Bcl-2 (not shown).

To test for dimerization between other Bcl-2 family members, each was cloned into the GAL4 DB and GAL4 AD vectors. Each GAL4 DB construct was tested with an empty GAL4 AD vector to detect any endogenous activation capacity within that construct. The Bcl-x_S (15) and A1 (20) inserts were limited by spurious activation (Table 1). An assessment of the various combinations of family members revealed a specificity of interactions (Table 1). Moreover, a quantitative ONPG assay (14) performed on standardized lysates assessed the strength of these protein interactions in the yeast two-hybrid system (Fig. 3). While this is not intended as a direct reflection of actual affinities within mammalian cells, it suggests a hierarchy in the strengths of interaction. A salient feature was the capacity of Bax to dimerize with multiple partners containing BH1 and BH2 domains, including Bcl-x_L, Mcl-1, and

Table 1. Interaction between Bcl-2 family members in GAL4 DB and AD vectors

		GAL4 AD						
		Empty	Bax	Bcl-2	Bcl-x_S	Bcl-x_L	Mcl-1	A1
GAL4 DB	Empty	ND	−	−	−	−	−	−
	Bax	−	+	+	−	+	+	+
	Bcl-2	−	+	+	+	−	−	+
	Bcl-x_S	+	ND	ND	ND	ND	ND	ND
	Bcl-x_L	−	+	−	+	−	−	−
	Mcl-1	−	+	−	−	−	−	−
	A1	+	ND	ND	ND	ND	ND	ND

The following constructs were analyzed: hu-Bcl-2ΔC22, mu-Bcl-2ΔC21, mu-BaxΔC18, hu-Bcl-x_LΔC19, hu-Bcl-x_SΔC19, mu-Mcl-1ΔC21, and mu-A1ΔC21. Similar results were obtained for mouse (mu-) and human (hu-) Bcl-2 constructs. The Bax GAL4 DB construct spuriously activated transcription by itself, which was circumvented by cloning Bax into the pBTM 116 vector encoding for a fusion protein specific for the LexA binding element in the L40 strain (13). The murine Mcl-1 cDNA was isolated by interactive λ expression cloning using Bax protein as a probe, providing independent confirmation of Mcl-1/Bax interaction. Interactions of Bcl-x_L and mu-Bcl-2 were also confirmed by analysis as LexA fusion proteins in pBTM 116. ND, not done.

A1. Bcl-x_S, which lacks BH1 and BH2, did not form a detectable interaction with Bax. The Mcl-1 (21) and A1 proteins scored the strongest with Bax in the yeast two-hybrid system; however, the spurious activating capacity of A1 may affect this result. Specificity exists in that many of the potential interactions between family members do not occur. For example, Mcl-1 displayed a marked selectivity only interacting with Bax. A1 appeared to also interact with Bcl-2, albeit less strongly than with Bax (Fig. 3). Bcl-x_S, which lacks BH1 and BH2, only interacted with the two death repressor molecules, Bcl-2 and Bcl-x_L (Table 1). Bcl-2 and Bax, which bear BH1 and BH2, also interacted with themselves. However, strong Bcl-x_L/Bcl-x_L or Mcl-1/Mcl-1 interactions were not detected (Fig. 3, Table 1). The overall pattern of interactions indicates that Bax has the widest capacity to dimerize with other family members containing BH1 and BH2.

To test whether interactions noted in the yeast two-hybrid system predicted meaningful dimerizations that would regulate apoptosis in mammalian cells, we selected the Bcl-x_L/Bax interaction for further study. Bcl-x_L is a known inhibitor of apoptosis (15). We generated a hamster anti-Bax mAb (4D2 mAb) that recognized mouse Bax (Fig. 4*A*, lanes 1 and 4) but not human Bax (Fig. 4*A*, lane 2) or other known family members (17). The 4D2 mAb also recognized a HA epitope-tagged p23 HA-Bax molecule (Fig. 4*A*, lane 5). The peptide sequence of the 21-kDa immunoprecipitated protein confirmed it was Bax. Of note, the 4D2 mAb also crossreacts with a p28 molecule that is not Bcl-x_L as determined by peptide sequence (Fig. 4, arrows). This was observed in RIPA buffer, which disrupts dimers of this family.

The interleukin 3 (IL-3)-dependent murine hematopoietic cell line FL5.12 has no substantial Bcl-x_L when assessed with a rabbit anti-Bcl-x hetero-Ab (15) (Fig. 4*B*, lane 1). When stably transfected clones of FL5.12 that overexpressed Bcl-x_L were immunoprecipitated with this Ab, a p21 species was coprecipitated in 0.2% Nonidet P-40 buffer. A Western blot of this immunoprecipitate developed with the 4D2 mAb confirmed that the p21 molecule was Bax (Fig. 4*B*, lane 2).

We next examined if the same sites that had dictated Bcl-2/Bax interaction were required for Bcl-x_L/Bax dimers. The WAR (mI-3) substitution that eliminated Bcl-2's heterodimerization with Bax (8) was introduced as Gly-159 → Ala within BH1 of Bcl-x_L (15). Immunoprecipitation of Bcl-x_L mI-3 within FL5.12 cells revealed that the amount of coprecipitated Bax was drastically reduced (Fig. 4*B*, lane 3). To confirm this, an epitope-tagged HA-Bcl-x_L mI-3 was immunoprecipitated with the 12CA5 anti-HA mAb. This mutant, when identified by the separate HA epitope, also failed to heterodimerize with Bax (Fig. 4*C*, lane 3). Following immunodepletion of Bcl-x_L, the remaining supernatant from FL5.12 Bcl-x_L mI-3 cells displayed abundant Bax compared to the supernatant from Bcl-x_L wt containing cells (not shown). Thus, when Bcl-x_L failed to dimerize with Bax, more Bax/Bax homodimers existed.

Of interest, the Bax epitope recognized by the 4D2 mAb is clearly present on Bax/Bax homodimers and Bcl-2/Bax heterodimers but less so on Bcl-x_L/Bax heterodimers. The 4D2 mAb coprecipitates the minimal amounts of Bcl-2 present in

DB-Bax	AD-A1	810	DB-Bcl-x_L	AD-Bcl-x_S	710
	AD-Mcl-1	220		AD-Bax	17
	AD-Bax	35		AD-Bcl-x_L	1.8
	AD-Bcl-2	26		AD-Bcl-2	1.5
	AD-Bcl-x_L	11		AD-Mcl-1	1.1
	AD-Bcl-x_S	1.1		AD-A1	0.8
	AD-empty	1.0		AD-empty	1.0
DB-Bcl-2	AD-Bcl-x_S	180	DB-Mcl-1	AD-Bax	37
	AD-Bax	30		AD-Bcl-x_S	1.4
	AD-Bcl-2	5.8		AD-Bcl-x_L	1.3
	AD-A1	2.6		AD-Mcl-1	1.3
	AD-Bcl-x_L	1.2		AD-A1	0.1
	AD-Mcl-1	1.0		AD-Bcl-2	0
	AD-empty	1.0		AD-empty	1.0

FIG. 3. Quantitation of β-galactosidase activity induced by protein–protein interactions. β-Galactosidase activity was determined by conversion of ONPG into *o*-nitrophenol, whose optical density was measured at 420 nm (14).

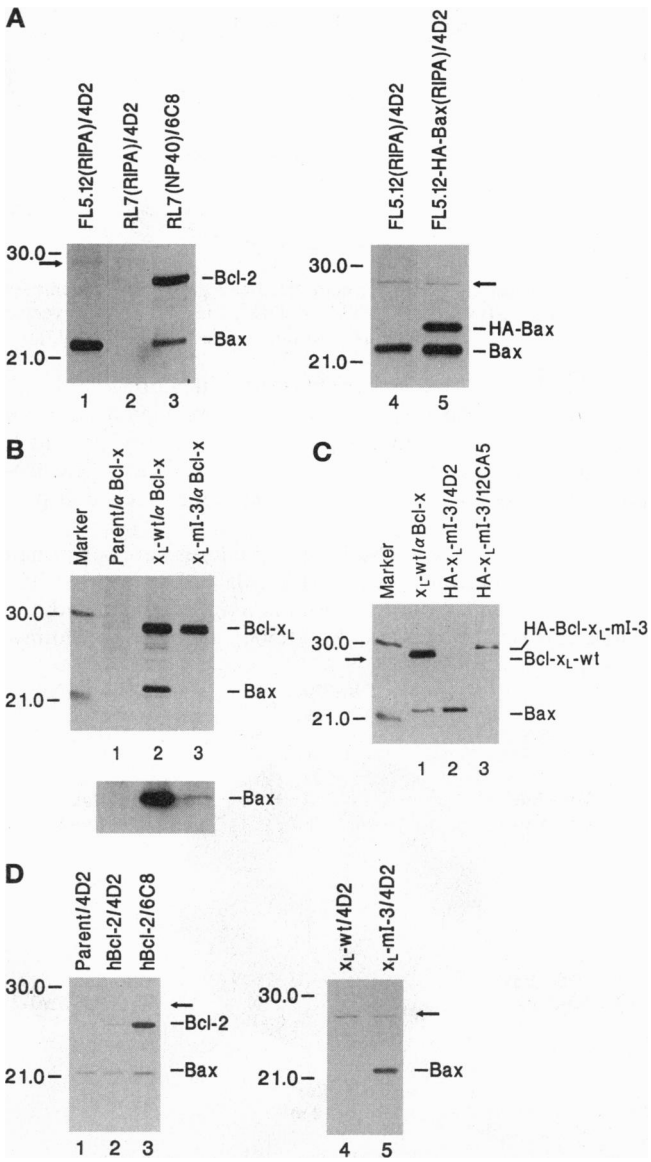

FIG. 4. The BH1 domain of Bcl-x_L is required for heterodimerization with Bax. (*A*) Cell lysates were prepared from ^{35}S-labeled murine FL5.12 cells, FL5.12 cells expressing HA-Bax, and RL-7, a human B-cell line bearing the t(14;18), which expresses high levels of Bcl-2 (22). Immunoprecipitation was performed with a hamster anti-mouse Bax mAb, 4D2 mAb, or the human Bcl-2-specific mAb, 6C8 (6). All immunoprecipitates were analyzed by SDS/PAGE. (*B Upper*) Cell lysates of ^{35}S-labeled FL5.12 cells (Parent) or stably transfected clones expressing Bcl-x_L wild-type (wt) or mI-3 (WAR) mutant were immunoprecipitated with a rabbit anti-Bcl-x hetero-Ab (α Bcl-x) (lanes 1–3). (*B Lower*) Western blot analysis of the above primary immunoprecipitates immunostained for Bax with the 4D2 mAb. (*C*) Cell lysates were prepared from ^{35}S-labeled FL5.12 stably transfected clones expressing Bcl-x_L wt or a hemagglutinin (HA)-tagged mutant with the WAR sequence (HA-x_L mI-3). Immunoprecipitations were performed with the anti-Bax 4D2 mAb, an anti-Bcl-x hetero-Ab (α Bcl-x), or the anti-HA epitope-specific mAb 12CA5 (23). (*D*) Cell lysates were prepared from ^{35}S-labeled FL5.12 (Parent) cells or stably transfected clones expressing human Bcl-2 (hBcl-2), Bcl-x_L wt, or the Bcl-x_L mI-3 mutant. Immunoprecipitation utilized the anti-Bax 4D2 mAb or the human Bcl-2-specific 6C8 mAb.

FL5.12 and substantially more Bcl-2 in FL5.12 cells overexpressing Bcl-2 (Fig. 4*D*, lanes 1 and 2). However, only a minimal amount of Bax homodimer was recognized by the 4D2 mAb in FL5.12 cells that overexpressed Bcl-x_L wt (Fig. 3*D*, lane 4). In contrast, Bax homodimers and the 4D2 Bax epitope were available in cells bearing the nondimerizing Bcl-x_L mI-3 or HA-Bcl-x_L mI-3 (Fig. 4*D*, lane 5, and 4*C*, lane 2). This indicates that the epitope availability varies between certain Bax dimers in these conditions.

To assess the functional significance of Bcl-x_L's interaction with Bax, a series of stably transfected FL5.12 clones was generated expressing either Bcl-x_L wt or Bcl-x_L mI-3 protein (Fig. 5*B*). When assessed for apoptosis following IL-3 deprivation all Bcl-x_L wt clones displayed extended cell survival. In contrast, Bcl-x_L mI-3 that failed to heterodimerize with Bax had also lost its death repressor activity (Fig. 5*A*).

DISCUSSION

A yeast two-hybrid system recapitulated the specificity of Bcl-2/Bax dimerizations known to be of functional significance in mammalian cells, justifying its use to search for further interactions. The interactions observed here suggest Bax may be a common partner involved in heterodimerization and regulation of the function of other family members (Fig. 6). Bcl-x_L was known to repress apoptosis and scored as a strong interaction with Bax in both orientations (Fig. 3). The assessment of Bcl-x_L/Bax in mammalian cells argues that other yeast two-hybrid predicted interactions may also prove to be functionally significant. A single amino acid substitution in BH1 disrupted Bcl-x_L's heterodimerization with Bax and abolished its inhibition of apoptosis. This argues that Bcl-x_L and Bcl-2 contact Bax in a similar fashion (8) and further emphasizes the importance of BH1 in survival function and the ability of family members to heterodimerize with Bax. Bcl-2 and Bcl-x_L heterodimerize with Bax, and this mutation analysis suggests that this heterodimerization is required for the repression of apoptosis. Thus, the role of Bcl-2 and Bcl-x_L could be to disrupt Bax/Bax homodimers. This might provide the connection between the death repressor and death effector pathways. Alternatively, each Bax heterodimer could represent a distinct, active moiety responsible for repressing cell death (Fig. 6).

The yeast two-hybrid analysis makes several other predictions. Bcl-x_S, which lacks BH1 and BH2, has been shown to promote cell death. Of note, Bcl-x_S countered protection by Bcl-2 (15). These findings may be explained by the selective interaction of Bcl-x_S with Bcl-2 as well as Bcl-x_L (Table 1, Fig. 3). This may effectively sequester the death repressor molecules of Bcl-2 or Bcl-x_L displacing them from Bax and thus restoring cell death. A recent study assessed the ratios of Bcl-2, Bcl-x, Bax, and a new partner, Bad, and correlated them with the death of IL-3-dependent FL5.12 cells. When approximately half or more of Bax was heterodimerized with either Bcl-2 or Bcl-x_L, apoptosis was inhibited (24). Thus, the spectrum of competitions of varied affinity noted in Fig. 6 would be expected to influence susceptibility to cell death.

Several potential interactions that were not detected in yeast two-hybrid analysis may also prove of significance. The DB-Bcl-x_L to AD-Bcl-x_L interaction only scored as a 1.8. We arbitrarily required interactions to be at least 2-fold above an empty AD vector background to be considered positive. In support of this, preliminary observations suggest that Bcl-x_L does not appear to form homodimers in mammalian cells (C.B.T., unpublished observations). Of note, the BH1 and BH2 mutants of Bcl-2 still interacted with Bcl-2 even though they were functionally inactive (8). This combined with the inability of Bcl-x_L to homodimerize argues against either Bcl-2 or Bcl-x_L homodimers being active moieties. An independent study predicted more widespread interactions of Bcl-2 family members including Bcl-x_L/Bcl-x_L and Mcl-1/Bcl-2 interactions (25). That system had not been validated with mutant control

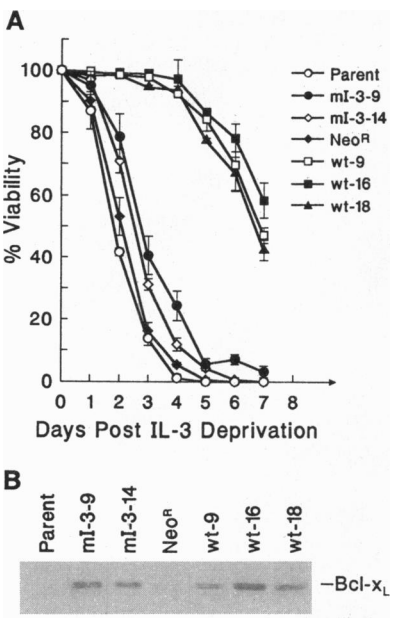

FIG. 5. Viability assays of Bcl-x_L wt and mutant clones. (*A*) Viability assays. Triplicate cultures of parent, neomycin-resistant (Neo^R) Bcl-x_L wt (wt-9, -16, -18), and Bcl-x_L mI-3 (mI-3-9, -14) expressing clones were deprived of IL-3. The percent viability was assessed by trypan blue exclusion at time points following IL-3 deprivation and plotted as the mean ± SE. (*B*) Western blot analysis of Bcl-x_L protein levels in FL5.12 parent, Neo^R, and Bcl-x_L wt (wt-9, -16, -18) and Bcl-x_L mI-3 (mI-3-9, -14) transfected FL5.12 clones. A Bcl-x_L-specific rabbit polyclonal hetero-Ab was used for immunostaining (15).

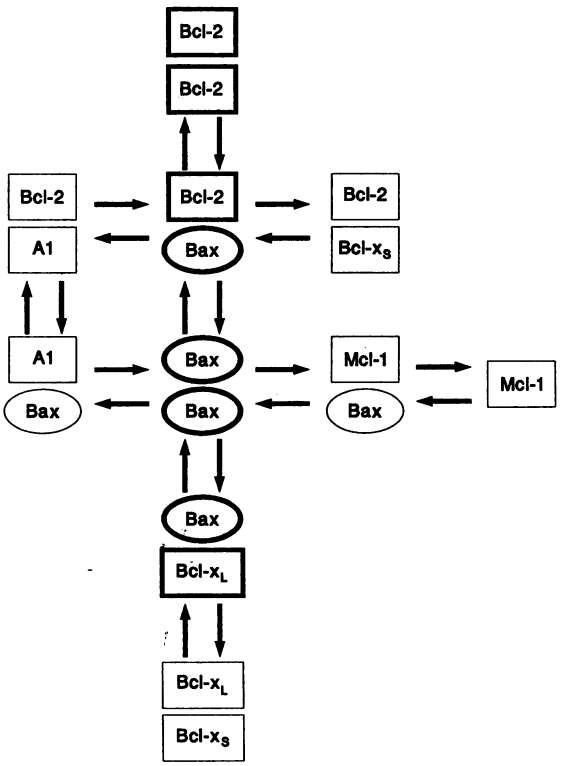

FIG. 6. Schematic model of protein–protein interactions based on the yeast two-hybrid system. Interactions proven to occur in mammalian cells are in bold outline. The exchanges indicated by the arrows are hypothetical.

proteins and was not quantitatively scored, though the authors analyzed human Mcl-1, whereas our study utilized a murine Mcl-1. A lethal effect of Bax was described in the EGY191 yeast strain (25); however, we have not observed any lethality or decreased transformation efficiency for Bax in L40, Y190, YPB2, or PCY2 yeast strains.

But why have so many family members? The loss of the 4D2 epitope in the Bcl-x_L/Bax heterodimer indicates that each dimer may be unique. Multiple family members would enable select interactions with other cytosolic proteins, perhaps linking cell death to other processes. In another system, a switch from Myc/Max to Mad/Max heterodimers coincides with a switch from cell proliferation to differentiation (26). We have noted some tissue specificity in the distribution of Bcl-2 family members and some members display reciprocal expression patterns during the differentiation of a single cell type (27, 28). However, certain cells such as dorsal root ganglion neurons express multiple members. In such instances the susceptibility to death, as proposed in Fig. 6, would reflect a complex set point determined by competing dimers of varying affinity.

We thank Stephen Elledge for the gift of the pASI-CYH2 and pACT II vectors. Pierre M. Chevray provided the yeast strain PCY2. The BTM 116 vector was constructed by Paul Bartel and Stanley Fields. Elaine Y. Lin and Michael B. Prystowsky graciously provided the A1 cDNA. We are indebted to Jennifer Jockel, Curt Milliman, and Xiao-Ming Yin for scientific contributions and to Mary Pichler for expert preparation of this manuscript. This work was supported by National Institutes of Health Grant CA 50239. L.H.B. is a fellow of the Leukemia Society of America. E.Y. is a Pfizer Fellow.

1. Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E. & Croce, C. M. (1985) *Science* **229,** 1390–1393.
2. Bakhski, A., Jensen, J. P., Goldman, P., Wright, J. J., McBride, O. W., Epstein, A. L. & Korsmeyer, S. J. (1985) *Cell* **41,** 899–906.
3. Cleary, M. L. & Sklar, J. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 7439–7443.
4. Vaux, D. L., Cory, S. & Adams, J. M. (1988) *Nature (London)* **335,** 440–442.
5. McDonnell, T. J., Deane, N., Platt, F. M., Nunez, G., Jaeger, U., McKearn, J. P. & Korsmeyer, S. J. (1989) *Cell* **57,** 79–88.
6. Hockenbery, D. M., Nunez, G., Milliman, C., Schreiber, R. D. & Korsmeyer, S. J. (1990) *Nature (London)* **348,** 334–336.
7. Oltvai, Z. N., Milliman, C. L. & Korsmeyer, S. J. (1993) *Cell* **74,** 609–619.
8. Yin, X.-M., Oltvai, Z. N. & Korsmeyer, S. J. (1994) *Nature (London)* **369,** 321–323.
9. Selvakumaran, M., Lin, H.-K., Miyashita, T., Wang, H. G., Krajewski, S., Reed, J. C., Hoffman, B. & Liebermann, D. (1994) *Oncogene* **9,** 1791–1798.
10. Miyashita, T., Krajewski, S., Krajewska, M., Wang, H. G., Lin, H. K., Liebermann, D. A., Hoffman, B. & Reed, J. C. (1994) *Oncogene* **9,** 1799–1805.
11. Fields, S. & Song, O. (1989) *Nature (London)* **340,** 245–246.
12. Nguyen, M., Miller, D. G., Yong, V. W., Korsmeyer, S. J. & Shore, G. C. (1993) *J. Biol. Chem.* **268,** 25265–25268.
13. Durfee, T., Becherer, K., Phang-Lang, C., Yeh, S.-H., Yang, Y., Kilburn, A. E., Lee, W.-H. & Elledge, S. J. (1993) *Genes Dev.* **7,** 555–569.
14. Rose, M. & Botstein, D. (1983) *Methods Enzymol.* **101,** 167–180.
15. Boise, L. H., Gonzalez-Garcia, M., Postema, C. E., Ding, L., Lindsten, T., Turka, L., Mao, X., Nunez, G. & Thompson, C. B. (1993) *Cell* **74,** 597–608.
16. Fuhlbrigge, R. C., Fine, S. M., Unanue, E. R. & Chaplin, D. D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5649–5653.
17. Schreiber, R. D., Hicks, L. J., Celada, A., Buchmeier, N. A. & Gray, P. W. (1985) *J. Immunol.* **134,** 1609–1618.
18. Guan, K. L. & Dixon, J. E. (1991) *Anal. Biochem.* **192,** 262–267.
19. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
20. Lin, E. Y., Orlofsky, A., Berger, M. S. & Prystowsky, M. B. (1993) *J. Immunol.* **151,** 1979–1988.
21. Kozopas, K. M., Yang, T., Buchan, H. L., Zhou, P. & Craig, R. W. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3516–3520.
22. Lipford, E., Wright, J. J., Urba, W., Whang-Peng, J., Kirsch, I. R., Raffeld, M., Cossman, J., Longo, D. L., Bakshi, A. & Korsmeyer, S. J. (1987) *Blood* **70,** 1816–1823.
23. Kolodziej, P. A. & Young, P. A. (1991) *Methods Enzymol.* **194,** 508–519.
24. Yang, E., Zha, J., Jockel, J., Boise, L. H., Thompson, C. B. & Korsmeyer, S. J. (1995) *Cell* **80,** 285–291.
25. Sato, T., Hanada, M., Bodrug, S., Irie, S., Iwama, N., Boise, L. H., Thompson, C. B., Golemis, E., Fong, L., Wang, H.-G. & Reed, J. C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 9238–9242.
26. Ayer, D. E. & Eisenman, R. N. (1993) *Genes Dev.* **7,** 2110–2119.
27. Veis, D., Sentman, C. L., Bach, E. A. & Korsmeyer, S. J. (1993) *J. Immunol.* **151,** 2546–2554.
28. Ma, A., Pena, J. C., Chang, B., Margosian, E., Davidson, L., Alt, F. W. & Thompson, C. B. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4763–4767.